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(54) Title: FLOCCULATION OF BIOLOGICAL MATERIAL FROM ORGANIC ACID-CONTAINING SYSTEMS			
(57) Abstract			
<p>The invention provides a process for the production of (meth)acrylic acid or salt thereof comprising providing (meth)acrylonitrile starting material and contacting it in an aqueous reaction mixture with an enzyme catalyst which comprises a nitrilase or a combination of nitrilehydratase and amidase and is provided in the form of free enzyme or free cells containing enzyme and allowing the catalyst to convert the (meth)acrylonitrile to (meth)acrylic acid or salt thereof, in which an anionic microparticulate material is mixed into the reaction mixture and flocculates the enzyme catalyst so that the flocculated enzyme catalyst can be separated from the aqueous methacrylic acid or salt thereof. In a further aspect, anionic microparticulate material can be used to flocculate biological material selected from cells, cell debris and enzyme from a reaction mixture having high conductivity and comprising organic acid or salt thereof, such as lactic acid.</p>			

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FLOCCULATION OF BIOLOGICAL MATERIAL FROM ORGANIC
ACID-CONTAINING SYSTEMS

This invention relates to processes for making aqueous solutions of ammonium (meth) acrylate and other (meth) acrylic monomers.

The production of (meth) acrylic acid and its salts, in particular ammonium (meth) acrylate, is industrially very important. These monomers are normally converted from (meth) acrylonitrile starting material. Chemical methods of achieving this conversion are known, but recently enzymic conversions have been developed. These may use for instance a nitrile hydratase to convert the (meth) acrylonitrile to (meth) acrylamide, followed by use of an amidase enzyme which converts the (meth) acrylamide to (meth) acrylic acid or its salts, usually ammonium (meth) acrylate. Alternatively a nitrilase enzyme may be used to convert the (meth) acrylonitrile directly to (meth) acrylic acid or salt thereof, normally ammonium (meth) acrylate.

One such process is described in our International Patent Publication W097/21827 (unpublished at the priority date of this application), in which very low levels of (meth) acrylonitrile are converted to very high levels of (meth) acrylic acid or salt thereof with the use of a nitrilase enzyme having very low K_m for (meth) acrylonitrile and very high K_i for ammonium (meth) acrylate.

In this and other enzymic processes for the production of ammonium acrylate and related monomers the enzyme catalyst may be provided in various forms. It may be present in purified free enzyme form or as part of a whole cell catalyst in free cell form. It is also known to provide whole cell catalysts in immobilised form in or on a support matrix.

Immobilised catalysts are very useful as a means of producing final product ammonium acrylate monomer uncontaminated with biological material such as cells, enzyme and cell debris. It is important to obtain such a

pure final monomer product, in particular in the case of ammonium acrylate and other polymerisable monomers because product contaminated with biological material tends to be of reduced quality and may even fail to polymerise successfully.

Immobilised biocatalysts are described for instance by Ashina and Suto in "Industrial Application of Immobilised Biocatalysts", 1993, Marcel Dekker, Chapter 7 and by Chaplin and Bucke in "Enzyme Technology", 1990, Cambridge University Press, pages 194 and 195. However, a disadvantage of immobilisation of cells is the fact that it results in a catalyst which is more complex to produce and is of increased catalyst cost. The cost may even be doubled by using immobilised catalyst.

In our Publication WO97/21827 we describe systems using free cells, in which cell material is removed by centrifugation and filtration from the final ammonium acrylate solution produced. Although such a method is acceptable, it can be inconvenient to obtain sufficient removal of cell debris and enzyme to ensure that the final product is of adequate purity.

Systems have been described for flocculation of cells from the fermentation broth in which they have been grown. One such system is described by Hughes et al in Biotechnology Techniques, vol. 4, number 4, 233-236 (1990). This article demonstrates flocculation of the bacteria Zymomonas mobilis and Bacillus subtilis from the medium in which they are grown using a charged high molecular weight polyelectrolyte followed by bentonite. The article specifically states that addition of bentonite prior to polyelectrolyte gave significantly inferior flocculation efficiency. Sitkey et al describe, in Acta Biotechnologica 12 (1992) 4, an alternative system for separation of cells from their fermentation broth. This system involves addition of bentonite to the cell-containing broth, followed by addition of flocculant during mixing of the broth.

We have found however that systems such as fermentation broth having a relatively low ionic strength behave very differently from those having a high ionic strength, such as ammonium acrylate solution, in particular when in high concentration. For instance we find that the system described by Hughes et al is ineffective in flocculating cells and enzyme from the final product ammonium acrylate solution.

Hughes et al report that it is known that separation of some cells from a fermentation broth is effective using a polymeric flocculant alone, whereas we find that this system is ineffective in the ammonium acrylate environment.

It would be desirable to provide an enzymically catalysed process for producing ammonium acrylate (or related monomers) which allows efficient and convenient separation of enzyme catalyst at the end of the reaction without the necessity to use immobilised enzyme catalyst with its attendant expense.

According to the invention we provide a process for the production of aqueous (meth) acrylic acid or salt thereof comprising providing (meth) acrylonitrile starting material and contacting it in an aqueous reaction mixture with an enzyme catalyst which comprises a nitrilase or a combination of nitrile hydratase and amidase and is provided in the form of free enzyme or free cells containing enzyme

and allowing the enzyme catalyst to convert the (meth) acrylonitrile to (meth) acrylic acid or salt thereof,

characterised in that the process also comprises mixing into the reaction mixture an anionic microparticulate material so as to flocculate the enzyme catalyst and separating the flocculated enzyme catalyst from the aqueous (meth) acrylic acid or salt thereof.

In the invention the anionic microparticulate material is added directly to the reaction mixture, that is no additional agglomerating agent such as polymeric flocculant is added before the anionic microparticulate material. We

find that, surprisingly, the addition of anionic microparticulate material directly to the reaction mixture gives rapid and efficient separation of cells, enzyme and cell debris from the reaction mixture, without the need to
5 carry out lengthy and inefficient filtration and centrifugation procedures. In particular, the use of anionic microparticulate material gives dramatically improved results in comparison with the use of synthetic flocculant alone. We also find surprisingly that a system
10 specifically stated by Hughes et al, in the Biotechnology Techniques reference discussed above, to be ineffective in flocculation of cells from culture broth is extremely effective in the specific environment produced at the end of a reaction for converting (meth) acrylonitrile
15 containing high levels of ammonium (meth) acrylate or other (meth) acrylic acid salt.

In this specification we define effectiveness of flocculation in terms of percentage reduction in turbidity (%RT). %RT is measured spectrophotometrically at 600 nm.
20 It is calculated as follows:

$$\%RT = \frac{A_1 - A_2}{A_1} \times 100\%$$

where A_1 = absorbance at 600 nm of unflocculated reaction mixture and A_2 = absorbance at 600 nm of flocculated
25 reaction mixture.

We consider that effective flocculation is carried out if the value of %RT is 50% or greater, and particularly 60 or 70% or greater. In the invention effective flocculation is particularly preferred to be carried out so as to give
30 %RT of 80 or 90% or greater and optimum systems can give %RT of at least 95% and even around 100%.

The %RT which is given for effective flocculation can vary according to the exact nature of the initial reaction mixture which is treated. For instance, in a reaction
35 mixture which has a very high initial absorbance, for instance above 20 or 25 at 600 nm, effective flocculation generally gives a final absorbance at 600 nm of below 0.5,

for instance below 0.3 or 0.1. For such a reaction mixture in which the initial absorbence is high, the %RT required to give the desirable final absorbence will be of the order of 90% or greater. However, for reaction mixtures which have a relatively low initial absorbence, for instance only about 1.0, the %RT required to give acceptable final absorbence may be lower, for instance of the order of 60 or 70%.

The invention can be applied to various different systems for the production of (meth) acrylic acid and salts thereof, in particular production of ammonium acrylate. It is preferred that it is applied to the systems which produce in the reaction mixture a high concentration of (meth) acrylic acid or salt thereof, in particular which produce a high concentration of ammonium acrylate. Preferably the concentration of product monomer is at least 10 or 20 wt%, preferably at least 25 or 30 wt%. It may be at least 35% and even up to 40 or 50 wt%.

A particularly preferred process to which the invention can be applied is a process in which we make an aqueous solution containing at least 30% by weight (meth) acrylic acid or salt thereof and below 0.2% (meth) acrylonitrile by a process comprising providing water and (meth) acrylonitrile in an amount sufficient to provide, upon hydrolysis, a concentration of (meth) acrylic acid or salt thereof of at least 30% by weight and providing during the process, in contact with the (meth) acrylonitrile, an enzyme for converting (meth) acrylonitrile to ammonium (meth) acrylate and which has K_m for (meth) acrylonitrile below 500 μM and K_i for ammonium (meth) acrylate above 100,000 μM , allowing hydrolysis of (meth) acrylonitrile to occur to provide a reaction solution which has a concentration of (meth) acrylonitrile of below 0.2 % and a concentration of ammonium (meth) acrylate of above 30 % and recovering a solution having concentrations of ammonium (meth) acrylate of above 30% and acrylonitrile of below 0.2%.

In this aspect of the invention we use an enzyme which can scavenge (meth) acrylonitrile to extremely low concentrations to produce ammonium (meth) acrylate even when the amount of ammonium (meth) acrylate or other (meth) acrylic acid salt present in the reaction solution can be very high. As a result we obtain a high concentration of ammonium (meth) acrylate or other (meth) acrylic acid monomer contaminated with only a very low amount of (meth) acrylonitrile. In particular, by using an enzyme which has a very low K_m value we are able to achieve a very low (meth) acrylonitrile content in the final product and by using an enzyme which also has a high K_i value we are able to achieve this low concentration of (meth) acrylonitrile in the presence of a high concentration of ammonium (meth) acrylate.

Such processes are described in our Publication WO97/21827 and the invention may be applied to any of the processes described therein.

In the process of the invention the reaction mixture is preferably subjected to a reaction stage, in which the (meth) acrylonitrile starting material is contacted with the enzyme catalyst and converted to (meth) acrylic acid or salt thereof. The reaction mixture is then subjected to a storage stage during which substantially no reaction takes place and in which the enzyme catalyst is separated from the aqueous solution of product monomer.

The process of the invention may for instance be a batch process. In such a process aqueous reaction medium (normally provided by water) enzyme catalyst and (meth) acrylonitrile starting material are placed in a reaction vessel and allowed to react such that the (meth) acrylonitrile is converted to (meth) acrylic final product monomer. This is the reaction stage. When sufficient reaction has taken place the reaction mixture enters the storage stage. This can take place in the same reaction vessel as the reaction stage but generally the reaction

mixture is removed from the reaction vessel and placed in a storage vessel.

In a batch process the final concentration of (meth) acrylic acid or salt thereof is often at least 10 wt%.

5 Alternatively the process of the invention may be a fed batch type process. In such a process (meth) acrylonitrile is fed into a reaction vessel in which the reaction takes place and in which has been provided aqueous reaction medium (usually water) and enzyme catalyst. In
10 the reaction stage the (meth) acrylonitrile is allowed to react to produce (meth) acrylic product monomer in such a way as to maintain the concentration of (meth) acrylonitrile between an upper and a lower concentration limit. The (meth) acrylonitrile may be fed continuously
15 into the reaction vessel to maintain a concentration within this range. Alternatively it may be fed to an extent that the concentration of (meth) acrylonitrile reaches the upper limit of the range. Feeding is then suspended until the concentration of (meth) acrylonitrile drops to the lower
20 limit of the range, at which point further feeding is commenced to raise the concentration of (meth) acrylonitrile again. This process is continued until sufficient conversion of (meth) acrylonitrile has taken place. Often this means the final concentration of (meth)
25 acrylic monomer is high, for instance at least 20 or 30 wt%. This is the end of the reaction stage.

The reaction mixture then passes to the storage stage. As with batch processes, the storage stage may be carried out in the reaction vessel in which the reaction stage took
30 place. Alternatively the reaction mixture may be transferred to a separate storage vessel.

A further process of the invention is a continuous process. In such a process (meth) acrylonitrile is fed into a reaction vessel which is also provided with enzyme
35 catalyst and aqueous reaction medium (normally water). The concentration of starting material and final product are maintained constant. Reaction mixture can be continuously

drawn off from the reaction vessel to give a product mixture which has the starting materials and product concentrations present in the reaction vessel. Usually the reaction mixture is drawn off at the same rate as the rate of feed of reactants, so as to maintain a fixed working volume in the reaction vessel.

Such a process is particularly advantageous when the concentration of (meth) acrylonitrile is maintained very low, for instance below 0.2%, often below 0.1%, preferably below 0.05% and the concentration of final product (meth) acrylic monomer is maintained at a high level, for instance at least 10 or even 20 or 30 wt%. The product which is drawn off therefore contains very low levels of (meth) acrylonitrile and very high levels of product (meth) acrylic monomer.

In such a process the reaction stage takes place in the reaction vessel. A single reaction vessel may be used. Alternatively a series of reaction vessels, through which the reaction mixture passes, may provide the reaction stage.

After the reaction stage the reaction mixture drawn off from the, or the final, reaction vessel is transferred to a storage stage. For instance, material drawn from the final reaction vessel may be supplied to a storage tank which gradually fills.

In the invention the anionic microparticulate material may be added to the reaction mixture after the reaction stage and during the storage stage. It is mixed into the reaction mixture so as to prevent premature agglomeration. This mixing may be carried out under low shear. Alternatively the anionic microparticulate material may be mixed into the reaction mixture at medium shear or high shear.

Mixing of the anionic microparticulate material into the reaction mixture may be carried out using an in-line mixer. For such systems in which high shear mixing is required the mixing preferably is such that the flow

through the mixer has a Reynold's number of at least 2,000. That is, the reaction mixture is passed through the in-line mixture so that it undergoes turbulent rather than laminar flow.

5 The anionic microparticulate material may also be mixed into the reaction mixture using other methods, for instance flow through a linear pipe or in a stirred vessel. In such systems also it is preferred that the reaction mixture and anionic microparticulate material are subjected
10 to high shear mixing. In particular, if the mixture is flowing through a pipe, it is subjected to turbulent flow so as to provide high shear mixing.

When the reaction mixture to which anionic microparticulate material has been added is subjected to
15 high shear, this is preferably shear equivalent to that applied under the following conditions:

200 ml of reaction mixture plus anionic
microparticulate material in a 400 ml beaker,
stirred using a 40 mm diameter Rushton turbine
20 impellor rotating at a rate of 300 rpm.

If mixing is to be carried out under low shear conditions it is preferably under conditions giving equivalent shear to a test using the above conditions but with an impellor speed of 50 rpm.

25 Additionally, after the anionic microparticulate material has been mixed into the reaction mixture in the storage stage a polymeric flocculant may be added. We find that this can give improved degree and rate of flocculation.

30 It is preferred that the anionic microparticulate material is subjected to medium or high shear after addition to the reaction mixture, in particular if polymeric flocculant is subsequently to be added.

The reaction mixture to which anionic microparticulate
35 material has been added may be sheared, if done, for any suitable time. If shearing is carried out during the storage stage it may be for at least about 2 minutes,

preferably at least about 4 or 5 minutes, for instance up to 10 minutes. The length of time of shearing will depend to a certain extent on the volume to be sheared. Generally shearing is carried out for sufficient time to ensure that the entire volume is subjected to the desired shear. A particularly preferred way of achieving this is by passing the entire volume of reaction mixture through an in-line mixer.

In some systems it is preferred that only anionic microparticulate material is added to the reaction mixture and no additional agglomerating agent such as polymeric flocculant is included.

In other processes of the invention the anionic microparticulate material may be mixed into the reaction mixture during the reaction stage. For instance, it may be stirred into the reaction vessel. In preferred processes the reaction mixture is cycled during the reaction stage by being drawn out of the reaction vessel via a loop and returned to the reaction vessel. Such a loop may be useful, for instance, for analysis of the state of the reaction. In particular a loop may be useful for addition of reactant such as (meth) acrylonitrile during the reaction stage, for instance in fed batch or continuous processes. In such a loop it is preferred that the material passes through an in-line mixer so as to ensure adequate mixing in of the material such as (meth) acrylonitrile being added.

If such a system is used the anionic microparticulate material may be added to the reaction mixture passing through the loop. This addition point is especially suitable for use in continuous processes. Preferably the reaction mixture plus anionic microparticulate material in the loop passes through an in-line mixer so as to ensure mixing of the anionic microparticulate material into the reaction mixture, often under high shear.

In continuous processes of the invention anionic microparticulate material may be added continuously or

repeatedly during the reaction stage. This may also be done in fed batch systems. However, in fed batch or batch systems it is preferred that a single dose of anionic microparticulate material is mixed into the reaction mixture prior to the reaction stage. That is, the anionic microparticulate material is mixed with some of the constituents of the reaction mixture, for instance water and catalyst. This is often done under low shear, but may be done under medium or high shear. This mixture is then charged to the reaction vessel. In a batch process the remaining constituents, for instance (meth) acrylonitrile, are then added and the reaction stage begins. In a fed batch process the remaining constituents, such as (meth) acrylonitrile, may be added gradually during the reaction. In the case of a fed batch process it is preferred that anionic microparticulate material is mixed under low shear with water and enzyme catalyst and this mixture charged to the reaction vessel. The (meth) acrylonitrile is then fed into the reaction mixture gradually via a re-circulation loop as discussed above. The anionic microparticulate material in the suspension is subjected to high shear as it passes through the in-line mixer which is provided in the recirculation loop.

It is preferred in processes in which the anionic microparticulate material is added during or prior to the reaction stage that no substantial flocculation of the enzyme catalyst occurs during the reaction stage. This can be achieved for instance by continually mixing the reaction mixture during the reaction stage. In a batch reaction this is conveniently achieved with the use of a batch stirred tank reactor. In a continuous or fed batch reactor this is conveniently achieved with the use of a continuous stirred tank reactor or a fed batch stirred tank reactor.

When the anionic microparticulate material is added to the reaction mixture during or prior to the reaction stage, it is often added at zero or very low concentration of (meth) acrylic acid or salt thereof. This occurs when for

instance anionic microparticulate material is mixed with water and enzyme catalyst before addition of (meth) acrylonitrile. The anionic microparticulate material does not induce flocculation of the enzyme catalyst under these conditions. However, as the level of (meth) acrylic acid or salts thereof increases throughout the reaction, flocculation begins.

On transfer to the storage stage the reaction mixture is subjected to conditions such that the enzyme catalyst is allowed to flocculate. This can be achieved for instance by simply allowing the mixture to rest. In preferred processes of the invention a polymeric flocculant is added to the reaction mixture during the storage stage. This assists in the flocculation. If desired the reaction mixture can be subjected to low, medium or high shear in the storage stage prior to addition of polymeric flocculant.

Separation of the flocculated enzyme catalyst may be carried out in any suitable manner. If fairly small flocs are formed (microflocs) these can be removed from the reaction mixture by filtration, for instance through a filter cloth. They may alternatively be separated by centrifugation.

Some processes produce larger flocs (macroflocs) which may be separated by settlement or flotation. Alternative methods of separation include filtration (under suction or under gravity), cross-flow filtration, centrifugation and use of a hydrocyclone.

The enzyme catalyst may comprise a mixture of nitrile hydratase, which acts to convert (meth) acrylonitrile to (meth) acrylamide, and amidase, which acts to convert methacrylamide to (meth) acrylic acid or salt thereof, normally ammonium (meth) acrylate. If this combination is used the two enzymes may be derived from the same microorganism or they may be derived from different microorganisms. Suitable amidases include those described in our International Patent Publication WO97/06248.

Preferably however the enzyme catalyst comprises a nitrilase which converts (meth) acrylonitrile directly to (meth) acrylic acid or salt thereof, normally ammonium (meth) acrylate. Microorganisms of the genus Rhodococcus often produce nitrile hydratase, nitrilase and/or amidase enzymes. Particularly suitable nitrilases are described in our copending International Patent Application published as WO97/21805 (unpublished at the priority date of this application). Especially good results are obtained using nitrilases produced by the Rhodococcus rhodochrous strains deposited under numbers NCIMB 40757 and NCIMB 40833 at the National Collections of Industrial and Marine Bacteria under the provisions of the Budapest Treaty. Strain NCIMB 40757 was deposited on 8 August 1995 and strain NCIMB 40833 was deposited on 11 December 1996. Subsequent to deposit of these strains we have found that they may be better characterised as Rhodococcus ruber.

The enzyme catalyst may be present in the reaction mixture in free enzyme form, the enzyme or enzymes having been extracted from the cells in which they were produced. Preferably however enzyme catalyst is present in free cell form. We find that this form, in particular in continuous processes, leads to improved stability of the enzyme or enzymes. Flocculation of free cells is particularly effective using the invention.

The enzyme catalyst may be included in the reaction mixture at any suitable point. In batch and fed batch reactions it is often included in the full amount at the beginning of the reaction, in mixture with water. In continuous processes this may also be done. However, in some continuous processes it is preferred to provide to the reaction stage a supply of fresh enzyme catalyst, added continuously throughout the reaction. This is normally supplied separately from anionic microparticulate material, if that is also added during the reaction stage. The enzyme catalyst does not usually undergo high shear, such as in an in-line mixer in a recirculation loop.

The process of the invention uses as starting material acrylonitrile or methacrylonitrile. Preferably it is acrylonitrile. The starting material is converted into acrylic acid or methacrylic acid or a salt thereof. Preferably it is ammonium acrylate.

The anionic microparticulate material is an agglomeration aid, that is it aids in inducing flocculation of the enzyme catalyst from the reaction mixture. It may be the same or similar to one of the anionic microparticulate retention aids known for use in the field of papermaking. It may be organic. For instance, anionic organic polymeric emulsions are suitable (especially microemulsions having a particle size mainly below $0.5 \mu\text{m}$, often below $0.2 \mu\text{m}$). The emulsified polymer particles may be insoluble due to being formed of a copolymer of, for instance, a water-soluble anionic monomer and one or more insoluble monomers such as ethyl acrylate, but preferably the polymeric emulsion is a cross-linked microemulsion of water-soluble monomeric material, for instance as described in US 5,167,766 and US 5,274,055 and commercialised under the trade name Polyflex.

Preferably it is inorganic, for instance colloidal silica (such as described in US 4,643,801), polysilicate microgel (such as described in EP-A-359,552), polysilicic acid microgel (such as described in EP-A-348,366), aluminium modified versions of any of these or, preferably, bentonite. In particular systems can be used as described in US 4,927,498, US 4,954,220, US 5,176,891 and US 5,279,807 and commercialised under the trade name Particol by Allied Colloids and Dupont.

The particle size of the microparticulate material is generally below $5 \mu\text{m}$, often below $2 \mu\text{m}$, preferably below $1 \mu\text{m}$ and most preferably below $0.1 \mu\text{m}$.

The preferred anionic microparticulate material is a swelling clay, generally a smectite, montmorillonite, sepiolite or hectorite. Suitable swelling clays are usually referred to as bentonites.

When bentonite is used as the anionic microparticulate material it is normally in the activated form which is generally used when bentonite is used in a retention system. That is, it is normally activated in conventional manner, so as to replace some of the calcium, magnesium or other polyvalent metal ions which are exposed, with sodium, potassium or other appropriate ions. Accordingly the bentonite is normally added to the reaction mixture as an aqueous slurry of sodium bentonite.

The anionic microparticulate material is normally added to the reaction mixture in amounts of from 3,500 ppm to 6,000 ppm, by weight based on total volume of reaction mixture. Preferably the amount is at least 4,000 ppm, more preferably at least 4,500 ppm. Particularly good results are obtained using a dose of at least 5,000, and in particular 5,500 ppm. Amounts above about 6,000 ppm tend not to improve results significantly beyond this. Amounts of from 100 to 2,000 ppm can also be used, eg 100 to 500 ppm.

We find that the level of anionic microparticulate material can affect the nature of the flocs finally formed. For instance, if anionic microparticulate materials such as bentonite is added at levels of around 200 ppm, for instance 100 to 400 ppm, the flocs formed tend to be small and can be removed by filtering. Higher levels, for instance 2,000 ppm and above, produce larger flocs which tend to float. The remaining reaction mixture may then be siphoned off.

The polymeric flocculant can be any polymeric material capable of improving the flocculation of the reaction mixture to which anionic microparticulate material has been added. It may be a naturally occurring polymeric material, for instance soluble starch or chitosan. Preferably it is a synthetic polymer, in particular one formed by polymerisation of ethylenically unsaturated monomer or monomer blend. The polymeric flocculant is normally provided as an aqueous solution.

The polymeric flocculant may be cationic, non-ionic or anionic. Normally it is of high molecular weight.

Preferred cationic polymeric flocculants have intrinsic viscosity at least 6 dl/g, for instance 8 to 15
5 dl/g or 8 to 20 dl/g or higher.

In this specification intrinsic viscosity is measured by suspended level viscometer at 25°C in 1N sodium chloride solution buffered to pH 7.

Suitable cationic polymers are copolymers of
10 ethylenically unsaturated cationic monomer, with the balance being other water-soluble, generally non-ionic, ethylenically unsaturated monomer such as acrylamide. The amount of cationic monomer is usually at least 2 or 3 mol%. Generally it is not more than 20 mol% but it can be up to
15 50 mol% or more.

The cationic polymer can be amphoteric, due to the inclusion of a lesser amount of anionic monomer, such as acrylic acid or other ethylenically unsaturated carboxylic monomer.

20 The polymer can be wholly water-soluble or it can be in the form of polymers which are cross-linked. The polymers may be made with a small amount of cross-linking agent, eg as described in EP-A-202,780. Preferred polymers of this type have an apparent IV of at least 3 dl/g and an
25 ionic regain of from 20 to 60%. The polymer may be linear.

Cationic polymeric flocculants preferably have a theoretical cationic charge density of not more than about 4 meq/g, often not more than about 3 or 2 meq/g. Often it is at least about 0.1, or usually at least about 0.5 meq/g.
30 In this specification, the theoretical cationic charge density is the charge density obtained by calculation from the monomeric composition which is intended to be used for forming the polymer.

Suitable cationic monomers include dialkylaminoalkyl
35 (meth) acrylates and -acrylamides as acid addition or quaternary salts. The alkyl groups may each contain 1 to 4 carbon atoms and the amino alkyl group may contain 1 to

8 carbon atoms. Particularly preferred are dialkylaminoethyl (meth) acrylates or acrylamides and dialkylamino-1,3-propyl (meth) acrylamides.

5 The polymer may be a cationic polymer of the type often referred to as a coagulant, eg poly diallyl dimethyl ammonium chloride.

10 In some systems the polymeric flocculant can be a copolymer of about 50 wt% diallyl dimethyl ammonium chloride and about 50 wt% acrylamide which has intrinsic viscosity at least 4 dl/g.

15 The polymer flocculant may be substantially non-ionic. In this case it is preferably a synthetic polymer having intrinsic viscosity above about 4dl/g and often above about 6dl/g, for instance up to 20 or 30 dl/g. It can be intended to be wholly non-ionic in which event it may be, for instance, polyethyleneoxide or polyacrylamide homopolymer (optionally including up to about 2 mol% sodium acrylate in the polymer) or it may be slightly anionic or slightly cationic. For instance it can contain up to 10 or 20 15 mol% anionic groups and up to 5 or 10 mol% cationic groups.

Preferred substantially non-ionic polymers are polymers having intrinsic viscosity of at least 4dl/g and formed of acrylamide alone or with up to 5 mol% cationic groups (preferably dialkylaminoalkyl acrylate or methacrylate quaternary salt) and/or with up to 8 mol% anionic groups (preferably sodium acrylate). Instead of using sodium acrylate, other water soluble acrylate salts or other anionic monomer groups can be used.

30 Suitable non-ionic flocculant polymers are described in our patent publications EP-A-608,986 and W095/02088. Other suitable non-ionic flocculant polymers are described in AU-A-63977/86.

35 The polymer flocculant may alternatively be an anionic polymer. Preferably it is of high molecular weight and has intrinsic viscosity at least 10, for instance at least 12 dl/g and even up to 20 or 30 dl/g.

Suitable anionic monomers from which anionic polymeric flocculant can be formed include ethylenically unsaturated carboxylic monomer such as acrylic acid, methacrylic acid and their salts, often polymerised together with acrylamide.

The polymer flocculant can be added in amounts of at least about 5 or 10 ppm up to about 100 or 200 ppm, by weight based on volume of reaction mixture. Amounts of from about 20 to about 50 ppm are preferred, for instance about 30 ppm.

We find that the use of the anionic microparticulate material in the invention allows for the use of lower levels of polymeric flocculant than are known for flocculation of biological material. In particular, the amount of polymeric flocculant in the invention is often below 70 ppm, preferably below 50 ppm, eg 5 to 35 ppm.

Cationic polymer flocculants are preferred as their levels can be particularly low. Additionally, they can give effective flocculation in combination with low levels of anionic microparticulate material, eg below 1,000 or 500 ppm, preferably below 300 ppm. Anionic polymer flocculants tend to be effective in combination with levels of anionic microparticulate material from around 500 ppm to 6,000 ppm, for instance 1,000 ppm and above.

We also find that the invention can be applied to other systems containing organic acid, which have high ionic strength. Thus in a second aspect of the invention we provide a method of separating biological material selected from cells, cell debris and enzyme from an aqueous reaction mixture having a conductivity of at least 20 mS and comprising an organic acid or salt thereof, comprising mixing into the reaction mixture an anionic microparticulate material, allowing the biological material to flocculate and separating the reaction mixture from the flocculated biological material.

Thus in this aspect of the invention we use anionic microparticulate material to flocculate cells, cell debris

or enzyme from a medium having high conductivity and a content of organic acid or (preferably) organic acid salt. In the past it has been found difficult to separate such biological materials from the defined reaction mixtures using conventional systems.

There are various circumstances in which it is found necessary to separate biological material such as cells, cell debris and enzyme from a high conductivity aqueous medium containing organic acid or salt thereof. This is necessary in some cases so that the biological material itself, for instance bacterial cells, may be recovered, for instance from a growth medium or from a system in which the cells have been carrying out a reaction and are required for re-use. Separation can also be required when for instance cells or enzyme have been carrying out a reaction to produce a particular end product which must itself be separated in a form free from contamination by biological material.

The growth media described in the Hughes et al article discussed above contain relatively low levels of ionic species and are of relatively low ionic strength. Similarly, the Sitkey et al article discussed above involves flocculation of cells from a medium having low ionic strength.

Thus this aspect of the invention provides an effective, economical and efficient method of separating biological material from the defined medium, for which conventional systems do not work.

In this aspect of the invention also the anionic microparticulate material is added directly to the reaction mixture, that is no additional agglomerating agent such as polymer flocculant is added before the anionic microparticulate material. We find that the method of this aspect of the invention is effective in flocculating biological material from the defined reaction mixtures, which have previously been considered to be "difficult".

The reaction mixture to which the invention is applied

has conductivity at least 20 mS, preferably at least 30 mS. In particular, suitable reaction mixtures may have conductivity 40 or 50 mS or greater, in particular at least 60 or 80 mS, and especially at least 100 mS. The conductivity may be up to 800 mS, but is normally not more than 700 or 600 mS.

For many reaction mixtures ionic strength is also high. Ionic strength is a measure of the concentration of ionic materials in an aqueous medium. It is calculated as half the sum of the products of the molarity of each ion with the square of its valency. For such mixtures the relationship between ionic strength and conductivity may be linear or non-linear.

Preferred reaction mixtures have high ionic strength, calculated using the formula set out above. In particular ionic strength is preferably at least 2.5, more preferably at least 3.0 or 3.5, often at least 4.0 or 5.0. It may be up to for instance 7.0 or 8.0.

The reaction mixture on which the method of this aspect of the invention may be practised may for instance be the result of a process of growing bacterial cells, which must then be separated from the growth medium. In the process the growth medium will contain high levels of ionic material including organic acid or salt, either because the bacteria require such an environment in which to grow or because they produce such materials during the course of their growth.

Alternatively and preferably the reaction mixture may be one which is the result of a fermentation process in which bacteria or enzyme are used to carry out a chemical conversion. The final product is often present in high concentration.

Preferred systems include those bioconversion processes giving a final product in high concentration which is an organic acid or salt thereof and which leads to high conductivity.

Examples include product streams of acidogenic yeasts, fungi and bacteria such as those which produce organic acids such as citric, glyconic, itaconic, 2-ketoglyconic, erythrobic, tartaric, lactic and acetic or amino acids such as L-glutamic acid, L-lysine, L-aspartic acid, L-tryptophan and D-arylglycines (or salts of any of these). Systems in which the invention is particularly useful are fermentation broths containing lactic acid bacteria, eg Lactobacillus species, which produce high concentrations of lactic acid in the reaction mixture, for instance at least 5 or 10 wt% lactic acid, up to 22 or 25 wt%.

In the method of this aspect of the invention biological material is separated from the reaction mixture. The biological material is selected from cells, cell debris and enzyme.

For instance, the reaction mixture may be the product of a reaction using enzyme catalyst to produce a final product such as lactic acid. In this case, free enzyme may be used as the catalyst. Alternatively, enzyme contained within cells or cell debris of the microorganism which produces it may be used as the enzyme catalyst, in which case the biological material to be separated comprises cells or cell debris.

The cells may be any appropriate cell, for instance yeast, algae, fungi or bacteria. For instance they may be bacterial cells grown in a growth medium having a high concentration of organic acid or salt thereof.

Preferably the biological material comprises cells, more preferably bacterial cells.

Reaction media of the type required in this aspect of the invention tend to be difficult to flocculate using conventional flocculation systems. The reaction mixture is often such that the flocculation performance of a cationic polymer formed from about 30 wt% dimethylaminoethyl acrylate (DMAEA) monomer and about 70 wt% acrylamide and having intrinsic viscosity of about 10 dl/g when used alone on the reaction mixture containing biological material is

not more than 50% of the flocculation performance of the same polymer acting on an equivalent suspension of biological material in water.

5 In preferred embodiments of this aspect of the invention, as in the first aspect of the invention, a polymer flocculant is also used to treat the reaction mixture in addition to the anionic microparticulate material. In this case the reaction mixture is preferably
10 flocculant when used alone on the reaction mixture containing biological material is not more than 50% of the performance of that polymer acting on an equivalent suspension of biological material in water.

15 In these tests, by an equivalent suspension we mean that the biological material is of the same type and is present in the same concentration, normally expressed as weight by volume of reaction mixture. The reference suspension is in water. It may be in pure water, but the test may also be carried out in water containing a very low
20 level of ionic or other materials which would at higher levels affect the nature of the reaction mixture so that polymeric flocculant alone is ineffective for separation of biological materials from the reaction mixture. For instance it may contain levels of such materials at about
25 the level of tap water.

The test suspension in water will normally have an ionic strength of about zero or close to zero. The suspension in water which is used for comparison with the reaction mixture normally has a low conductivity, for
30 instance below about 1 mS, usually below 0.5 mS, often about 0.2 mS.

In this aspect of the invention it is essential that an anionic microparticulate material is added to the reaction mixture. It may be added in any of the ways and
35 amounts discussed above in connection with the first aspect of the invention and may be any of the materials discussed in connection with the first aspect of the invention.

In this aspect of the invention also it is possible to add anionic microparticulate material at the beginning of a reaction, during which it does not cause agglomeration of the biological material, but as the reaction proceeds and conductivity and concentration of acid or salt build up the flocculation begins to occur.

Preferably a polymer flocculant is added to the reaction mixture after the anionic microparticulate material, usually after any reaction is finished. This may be any of the polymers and be added in any of the amounts discussed above in connection with the first aspect of the invention.

The invention will now be illustrated with reference to the following examples.

Examples

In the following examples the bacteria R.Rhodochrous strain NCIMB 40757 or 40833 are used.

Example 1 (Reference)

Acrylonitrile is fed into a suspension of R. rhodochrous in water for 19 hours. After completion of the reaction batch the product suspension consists of 40% (w/w) ammonium acrylate, 13 g/l R.Rhodochrous and < 10 ppm acrylonitrile. Filtration of the cells/monomer mixture through a 5 μ m filter cloth fails to retain the cells. Standing the cell/monomer mixture for 15 minutes results in no cell settlement.

Example 2 (Comparative)

Addition of high, medium or low molecular weight cationic, anionic or non-ionic synthetic flocculants to the cell monomer mixture of Example 1 fails to cause cell flocculation as evidenced by failure of the 5 μ m filter cloth to retain cell flocs and standing to cause separation by settlement of cell flocs.

Example 3 (Comparative)

Addition of the particulate filtration aid dicallite - a diatomaceous earth silicate material - to the cell/monomer product mixture of example 1, followed by

filtration through a 5 μ m filter cloth fails to separate the cells and other colloidal matter from the 40% ammonium acrylate product of Example 1.

Example 4 (Comparative)

When the cell/monomer mixture of Example 1 is passed through a 5 μ m filter cloth which has been pre-coated with dicalite, a very small amount of clarified product is obtained before the 5 μ m filter cloth is blinded by the biological colloidal matter.

Example 5

Addition of 5500 ppm of hydrated sodium bentonite to the cell/monomer mixture of Example 1 under low shear conditions (as defined below in Example 8) results in the formation of cell/bentonite flocs which settle under gravity over 15 minutes to give a monomer solution of high clarity. When the cell/bentonite/monomer mixed suspension is filtered through a 5 μ m filter cloth under a pressure difference of 28 inches of mercury (13.7 psi) the clarity of the filtrate was high (Absorbance at 600 nm = < 0.1) although the filtration rate was low (1.5 ml/min).

Example 6

If a synthetic polymer flocculant such as Polymer A, a cationic flocculant formed from 30 wt% dimethylaminoethyl acrylate quaternised with MeCl and 65 wt% acrylamide and of IV 11 to 12 dl/g, is added at a dose of 30 ppm under low shear (as defined below in Example 8) to the cell/bentonite/monomer mixture described in Example 5, macroflocs are formed. The settlement rates and filtration rates for the suspensions from Examples 5 and 6 are compared in Table 1.

Table 1

Suspension	Appearance	Relative Settlement Rate	Relative Filtration Rate	%RT
Cells/ bentonite/ monomer	Flocs	1	1	100
Cells/ bentonite/ Polymer A/ monomer	Macro Flocs	70	20	100

$$\% RT = \frac{A_1 - A_2}{A_1} \times 100\%$$

Where A_1 = Absorbance @ 600 nm of original cell/monomer suspension

Where A_2 = Absorbance of cell/monomer suspension after bentonite and/or flocculant treatment and settlement for 15 minutes.

Example 7

Table 2 shows the effect on % RT when different doses of bentonite and zero and 30 ppm of Polymer A are added under the low shear conditions described in Example 8 below.

Table 2

Bentonite Dose (ppm)	% RT	% RT after 30 ppm Polymer A
1220	16	20
2381	16	23
3488	43	44
4545	62	68
5556	100	100

It can be seen from Table 2 that there is an optimum dose of bentonite which is required to clarify most effectively the 40% (w/w) ammonium acrylate upon settlement for 15 minutes i.e. an amount of 5556 ppm gives 100% RT. Addition of synthetic polymer has little effect on the clarity of the settled monomer mixture but does increase the settlement rate.

Example 8

Example 7 shows that when the cell/monomer mixture from Example 1 is mixed with a lower than optimum dose of bentonite under low shear conditions then less than 100% RT is achieved upon settlement for 15 minutes. However, when high shear is applied to a sub-optimum bentonite dose such as 3500 ppm for 6 minutes a %RT of 100% is observed. For this example low shear is defined as: a 40 mm diameter Rushton turbine impellor rotating at a rate of 50 rpm in a 400 ml beaker of 200 ml of suspension, and high shear is defined as the above conditions with an impellor speed of 300 rpm.

Example 8 shows that by shearing down a cell/monomer/bentonite mixture the dose of bentonite required to give 100% clarity is reduced.

Example 9

To 200 g of cell/monomer mixtures as described in Example 1, is added hydrated bentonite at a dose of 2000 ppm. under the low shear mixing conditions of Example 8 ie in a stirred tank reactor. The mixtures are then passed through an in-line static mixer at different flow rates to produce different levels of shear in the static mixer. The mixture is allowed to settle for 15 minutes and the % RT measured. Where 100% RT is achieved the filtration rate when 200 g of the mixture is passed through a 5 μ m filter cloth as described in Example 5 is determined as shown in Table 3.

Table 3

Reynolds No	% RT	Filtration Rate (ml/min)
720	47	-
947	90	-
1227	100	1.5
2220	100	4.3

Example 10

The cell/bentonite mixture described in Example 1 is passed repeatedly from a stirred tank reactor through an in-line static mixer as described in Example 9 at a flow rate giving a Reynolds number of 2200. Table 4 shows the effect of mixing time on the % RT of the settled cell/bentonite mixture.

Table 4

In line mixing time (mins)	% RT
1	52
2	94
4	99
6	100
8	100

Example 11

When a cell/bentonite/monomer mixture is sheared at a Reynolds number of 2220 as described in Example 9, the filtration rate of the mixture through a 5 μ m filter cloth can be enhanced by the addition of a high molecular weight cationic (Polymer A) or anionic (Polymer B, formed from 10 wt% sodium acrylate and 90 wt% acrylamide and of IV 20 dl/g) flocculant but not a low molecular weight cationic polymer (Polymer C, a polydiallyl dimethyl ammonium chloride of IV 0.3 dl/g) which causes floc redispersion as shown by Table 5.

Table 5

Bentonite (ppm)	Filt, Rate (FR) (ml/min)	FR + Polymer A (ml/min)	FR + Polymer B (ml/min)	FR + Polymer C (ml/min)
2000	2	30	4	Turbid
2500	6	33	7.5	Supernatant
3500	6	27	40	

Example 12

R rhodochrous NCIMB 40757 cell paste 26 g is mixed with 19 g of water. A 5% (w/w) bentonite suspension (31 g) is mixed under high shear with 50 g of water. The R
5 rhodochrous cell suspension and the bentonite suspension are then mixed in a vessel and the cell/bentonite/water mixture is then added to 450 g of water in a stirred tank reactor. Over a 19 hour period or a 7 hour period acrylonitrile is added to the reactor. The acrylonitrile
10 hydrolysed to ammonium acrylate by the R rhodochrous nitrilase enzyme under the high shear conditions of the stirred tank reactor until an ammonium acrylate concentration of 40% (w/w) is achieved. The synthetic polymer flocculant Polymer A is then added to the
15 cell/bentonite/monomer solution and macroflocs are formed. The macroflocs are then retained in a 5 μ m filter cloth whilst the filtrate is a 100% clarified 40% ammonium acrylate solution.

Example 13

20 A cell/bentonite/water mixture as described in Example 12 is prepared in a stirred tank reactor modified by the addition of a recirculation loop through which the contents are pumped out from the base of the reactor through a in-line static mixer at a flow rate which gives a Reynolds
25 number of > 2000 and back into the reactor. Acrylonitrile is fed into the recirculation loop at the point of maximum shear (which is at the in-line static mixer) over a 19 or 7 hour period and is converted to ammonium acrylate until an ammonium acrylate concentration of 40% (w/w) is
30 achieved. During the 19 to 7 hour period the contents of the reactor are circulated through the recirculation loop around 20 times. Addition of synthetic polymer flocculation directly to the reactor under low shear induces the formation of macroflocs. The macroflocs are
35 then retained in a 5 μ m filter cloth whilst the filtrate is a 100% clarified 40% ammonium acrylate solution.

Example 14

Macroflocs of cells/bentonite/polymer formed as in Examples 6 to 13 can be readily separated to yield a 100% clarified 40% ammonium acrylate solution by: filtration under gravity, cross-flow filtration, centrifugation, settlement, hydrocyclone or flotation.

Example 15

This test was carried out on Lactococcus species fermentation broth, which has a very high ionic strength due to the high concentration of lactic acid. Flocculation was achieved by vortex mixing for 5 seconds aliquots of 2% sodium bentonite suspension with the broth. Vortex mixing was carried out at 500 rpm for 2 minutes in a Minstral 2000 centrifuge. Aliquots of 0.1% polymer E, a polydiallyldimethyl ammonium chloride polymer, were then added and also vortex mixed for 5 seconds. Results are shown in Table 1 below.

Table 1 %RT Data for Broth Treated Sequentially with Bentonite and Polymer E

Polymer E Dose (mg/l)	Bentonite Dose (mg/l)	800	1,600	2,400
20		81.76	83.41	86.56
60		79.48	80.35	86.4
100		75.79	75.47	85.61

From Table 1 the optimum dose of bentonite is 2400 mg/l followed by 20 mg/l Polymer E.

CLAIMS

1. A process for the production of aqueous (meth) acrylic acid or salt thereof comprising providing (meth) acrylonitrile starting material and contacting it in an aqueous reaction mixture with an enzyme catalyst which comprises a nitrilase or a combination of nitrile hydratase and amidase and is provided in the form of free enzyme or free cells containing enzyme

and allowing the enzyme catalyst to convert the (meth) acrylonitrile to (meth) acrylic acid or salt thereof,

characterised in that the process also comprises mixing into the reaction mixture an anionic microparticulate material so as to flocculate the enzyme catalyst and separating the flocculated enzyme catalyst from the aqueous (meth) acrylic acid or salt thereof.

2. A process according to claim 1 which comprises a reaction stage during which the enzyme catalyst converts the (meth) acrylonitrile to aqueous (meth) acrylic acid or salt thereof and a storage stage during which the enzyme catalyst is separated from the aqueous (meth) acrylic acid or salt thereof, in which the anionic microparticulate material is mixed into the reaction mixture during the reaction stage and is allowed to flocculate the enzyme catalyst during the storage stage.

3. A process according to claim 2 comprising cycling the reaction mixture during the reaction stage by drawing reaction mixture off via a loop and adding anionic particulate material to the reaction mixture in the loop and passing the reaction mixture through an in-line mixer in the loop.

4. A process according to claim 1 which comprises a reaction stage during which the enzyme catalyst converts the (meth) acrylonitrile to aqueous (meth) acrylic acid or salt thereof and a storage stage during which the enzyme catalyst is separated from the aqueous (meth) acrylic acid or salt thereof, in which the anionic microparticulate material is mixed into the reaction mixture prior to the

reaction stage and is allowed to flocculate the enzyme catalyst during the storage stage.

5. A process according to any of claims 2 to 4, comprising adding to the reaction mixture during the storage stage a polymeric flocculant.

6. A process according to any of claims 2 to 5 comprising mixing the reaction mixture in the reaction stage after addition of anionic microparticulate material or in the storage stage under high shear.

7. A process according to claim 1 which comprises a reaction stage during which the enzyme catalyst converts the (meth) acrylonitrile to (meth) acrylic acid or salt thereof and a storage stage during which the enzyme catalyst is separated from the (meth) acrylic acid or salt thereof, in which the anionic microparticulate material is mixed into the reaction mixture during the storage stage.

8. A process according to claim 7 which comprises adding to the reaction mixture after addition of anionic microparticulate material a polymer flocculant.

9. A process according to claim 7 or claim 8 comprising subjecting the reaction mixture to high shear after addition of anionic microparticulate material and before addition of polymeric flocculant.

10. A process according to any of claims 2 to 9 in which the reaction stage is a fed batch process and the reaction mixture is transferred to the storage stage at the end of each batch.

11. A process according to any of claims 2 to 9 in which the reaction stage is a continuous process and reaction mixture is continually drawn off from the reaction stage and transferred to storage vessels for the storage stage.

12. A process according to any preceding claim in which the starting material is acrylonitrile and is converted to ammonium acrylate and the ammonium acrylate is present in the storage stage at a concentration of at least 15 wt%, preferably at least 30 wt%.

13. A process according to any preceding claim in which the catalyst is provided in the form of free cells containing enzyme.

14. A process according to any preceding claim in which the enzyme catalyst comprises a nitrilase and is provided in the form of free cells containing the nitrilase.

15. A process according to any of claims 2, 3, 4 and 6 in which the anionic microparticulate material is the only agglomeration aid added to the reaction mixture.

16. A method of separating biological material selected from cells, cell debris and enzyme from an aqueous reaction mixture having a conductivity of at least 20 mS and comprising organic acid or salt thereof,

comprising mixing into the reaction mixture an anionic microparticulate material, allowing the biological material to flocculate and separating the reaction mixture from the flocculated biological material.

17. A method according to claim 16 in which the reaction mixture has a conductivity of at least 50 mS.

18. A method according to claim 16 or claim 17 which is a fermentation process in which bacteria or enzyme are used to produce organic acid or salt thereof as a final product.

19. A method according to any of claims 16 to 18 in which the organic acid or salt thereof is lactic acid or salt thereof.

20. A method according to any of claims 16 to 19 in which the reaction mixture has an ionic strength of at least 3.0.

21. A method according to any of claims 16 to 20 which is a process for production of a final product material catalysed by enzyme contained within the biological material.

22. A process according to any preceding claim in which the anionic microparticulate material is selected from anionic organic polymeric microemulsions having particle size mainly below 0.5 μm , colloidal silica, polysilicate microgel, polysilicic acid microgel and swelling clays.

33

23. A process according to any preceding claim in which the anionic microparticulate material is bentonite.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01806

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12P7/40 C12N1/02 C12P1/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 444 640 A (HIDEAKI YAMADA ;NITTO CHEMICAL INDUSTRY CO LTD (JP)) 4 September 1991 see page 7; table 3 see claims	1
Y	EP 0 325 348 A (ICI PLC) 26 July 1989 see the whole document	1
Y	US 3 278 391 A (H.W.RUELIUS) 11 October 1966 see claims	1
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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INTERNATIONAL SEARCH REPORT

Int. :ional Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE WPI Section Ch, Week 9650 Derwent Publications Ltd., London, GB; Class D15, AN 96-500369 XP002080888 & JP 08 256782 A (AGENCY OF IND SCI & TECHNOLOGY), 8 October 1996 see abstract</p> <p>-----</p>	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

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